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(54) Title: METHOD OF INHIBITING PHOTOAGING OF SKIN (57) Abstract Photoaging of undamaged skin due to UVB irradiation exposure is inhibited by administering an agent that inhibits at least one of (1) the activity of UVB irradiation inducible MMPs in the skin, (2) one or both of the transcription factors AP-1 and NF- B or (3) at least one of the GTP binding proteins or kinases involved in the activation and/or production of jun or fos proteins that comprise AP-1; and topically administering said inhibitor to the skin prior to such exposure.		

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METHOD OF INHIBITING PHOTOAGING OF SKIN

TECHNICAL FIELD

This invention is in the field of photoprotection. More particularly it relates to a method for inhibiting photoaging of undamaged skin using inhibitors of matrix metalloproteinase (MMP) production and/or activity.

BACKGROUND

5 Photoaging is a term used to describe the changes in appearance and function of skin as a result of repeated exposure to sunlight. The ultraviolet (UV) component of sunlight, particularly middle UV (called UVB, 290-320nm
10 wavelength) is the principal causative agent of photoaging. The extent of UVB exposure required to cause photoaging is not currently known. Repeated exposure to UVB at levels that cause erythema and tanning are, however, commonly associated with photoaging. Clinically,
15 photoaging is characterized by coarseness, wrinkling, mottled pigmentation, sallowness, laxity, telangiectasia, lentigines, purpura and easy bruising, atrophy, fibrotic depigmented areas, and ultimately premalignant and malignant neoplasms. Photoaging commonly occurs in skin
20 that is habitually exposed to sunlight such as the face, ears, bald areas of the scalp, neck, and hands.

Procedures for preventing photoaging of unaged skin and treating already photoaged skin are available. Sunscreens are commonly used to prevent photoaging of skin
25 areas that are habitually exposed to sunlight. Sunscreens are topical preparations that absorb, reflect, or scatter UV. Some are based on opaque particulate materials such as zinc oxide, titanium oxide, clays, and ferric chloride. Because such preparations are visible and occlusive, many
30 people consider these opaque formulations cosmetically unacceptable. Other sunscreens contain chemicals such as p-aminobenzoic acid (PABA), oxybenzone, dioxybenzone,

ethylhexyl-methoxy cinnamide and butylmethoxydibenzoylmethane that are nonopaque and colorless because they do not absorb light of visible wavelengths. While these nonopaque sunscreens may be more acceptable cosmetically they are still relatively short-lived and susceptible to being removed by washing or perspiration. Additionally all sunscreens reduce vitamin D production.

Rieger, M.M. *Cosmetics and Toiletries* (1993) 108:43-56 reviews the role of reactive oxygen species (ROS) in UV-induced aging of skin. This article reports that topical application of known antioxidants to the skin can reduce the presence of ROS in the skin and thus reduce photodamage.

Retinoids have been used to retard the effects of photoaging in sun-damaged skin. U.S. Pat. No. 4,877,805 describes the treatment of photoaged skin as intervention therapy to decelerate the photoaging process. The patent indicates that there is little point in beginning such treatment until the effects of aging begin to appear. In this regard the present applicants know of no art that suggests the use of retinoids to prevent photoaging of undamaged skin.

MMPs are a family of enzymes that play a major role in physiological and pathological destruction of connective tissue. Over 10 members of the family have been identified. They are referred to numerically (MMP-1, MMP-2, etc.) as well as by common name. They appear to share several structural and functional properties but differ in their tissue substrate specificities. They include interstitial collagenase (MMP-1) and PMN-collagenase (MMP-8) that degrade collagen types I, II, III, VII, VIII, IX, and gelatin; the 72kDa (MMP-2) and 92kDa (MMP-9) type IV collagenases/gelatinases that degrade collagen types IV, V, VII, X, XI, gelatin, elastin, and fibronectin; stromelysin-1 (MMP-3),

stromelysin-2 (MMP-10), and stromelysin-3 (MMP-11) that degrade fibronectin, PG core protein, collagen types IV, V, IX, and X, laminin and elastin; PUMP-1 (MMP-7) that degrades collagen type IV, gelatin, laminin, fibronectin and PG core protein; and metalloelastase (MMP-12) that degrades elastin and fibronectin.

The expression of MMP genes is induced by the transcription factors AP-1 and NF- κ B. Angel, P. et al., Cell (1987) 49:729-739 and Sato, H. and Seiki, M., Oncogene (1993) 8:395-405. AP-1 and NF- κ B activities are mediated by cytokines (e.g., interleukins IL-1, IL-6, and TNF α), growth factors (TGF α , bFGF), and environmental stress such as oxidants, heat, and ultraviolet irradiation. AP-1 induction and production of jun proteins (C-jun, jun-B, and jun-D) and fos proteins (C-fos, fos-B, fra-1, and fra-2) that make up AP-1 are mediated by a host of molecules (e.g. RAC, CDC42, MEKK, JNKK, JNK, RAS, RAF, MEK, and ERK). It is known that AP-1 and NF- κ B are activated in mammalian cells exposed to UV light. Devery, Y., et al. Science (1993) 261:1442-1445. Wlaschek, M. et al., Photochemistry and Photobiology (1994) 59(5):550-556, also report that UVA irradiation of fibroblasts resulted in an IL-1 and IL-6-mediated induction of MMP-1 and that such induction might contribute to the loss of collagen in photoaging.

Inhibitors of MMPs or the transcription factors that affect their expression are also known. Hill, P.A. et al., Biochem J (1995) 308: 167-175 describes two MMP inhibitors, CT1166 and RO31-7467. Gowravaram, M.R. et al., J Med Chem (1995) 38:2570-2581 describes the development of a series of hydroxamates that inhibit MMPs and mentions thiols, phosphonates, phosphinates, phosphoramidates and N-carboxy alkyls as known MMP inhibitors. This paper indicates that MMP inhibitors include a moiety that chelates zinc and a peptidic fragment that binds a subset of the specificity pockets of

MMPs. Hodgson, J., Biotechnology (1995) 13:554-557 reviews the clinical status of several MMP inhibitors, including Galardin, Batimastat, and Marimastat. Other MMP inhibitors include butanediamide (Conway, J.G. et al., J Exp Med (1995) 182:449-457), TIMPs (Mauch C., et al., Arch Dermatol Res (1994) 287:107-114), and retinoids (Fanjul, A. et al., Nature (1994) 372:107-111; Nicholson, R.C. et al., EMBO Journal (1990) 9(13) 4443-4454; and Bailly, C. et al., J Investig Derm (1990) 94(1):47-51).

10 DISCLOSURE OF THE INVENTION

The present invention is based on applicants discovery that UVB exposure rapidly upgrades AP-1 and NF-B in the exposed skin and leads to MMP induction. The elevated levels of MMPs that result from UVB exposure act to degrade connective tissue proteins in skin. Such damage, if imperfectly repaired, results in solar scars which accumulate through repeated UVB exposure and also cause photoaging.

Accordingly, applicants prevent photoaging of undamaged human skin due to exposure of the skin to UVB by administering an inhibitor of a UVB-inducible MMP to the human prior to said exposure in an amount sufficient to inhibit induction and/or activities of UVB-inducible MMPs. Surprisingly, this occurs at UVB doses below those that cause erythema as well as at those which cause erythema.

Another aspect of this invention is the use of an inhibitor of UVB-inducible MMP induction or activity in the manufacture of a medicament for preventing photoaging of undamaged skin due to repeated UVB exposure.

30 BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings:

Fig. 1 is a flow chart showing the pathways by which UVB induces MMP production.

Figs. 2a-d, 3a-b, 4a-d, and 5a-e are graphs of test results described in the Examples, infra.

MODES FOR CARRYING OUT THE INVENTION

The present invention is used to inhibit (i.e. reduce or prevent) photoaging of undamaged human skin, that is, skin that does not show the effects of photoaging.

Treatment according to this invention should thus be practiced on skin such as that of the head, neck, hands, and arms that in typical, everyday living are habitually exposed to sunlight before such skin exhibits the telltale signs of photoaging. Because repeated exposure to doses of UVB below that which causes erythema can lead to photoaging, the invention should be practiced on skin subject to such low dose exposure. In this regard UVB doses in the range of 30-50 mJ/cm² skin cause erythema in most fair-skinned people. Accordingly the invention will prevent photoaging of skin subjected to doses below this range (typically above about 5 mJ/cm² which is equivalent to a few minutes of sunlight exposure).

Photoaging is prevented or inhibited according to the invention by inhibiting UVB-induced degradation of the dermal extracellular matrix by MMPs. This is accomplished by administering a MMP inhibitor to the skin that is to be exposed to sunlight. In this regard the term "MMP

inhibitor" intends those agents that directly or indirectly inhibit (i.e., reduce significantly or eliminate) the expression of UVB-inducible MMPs in such skin or inhibit the enzymatic activity of such MMPs.

"Indirect inhibition" is intended to mean interaction with either or both of the transcription factors AP-1 and NF- κ B and/or one or more of the molecules involved in the three kinase cascades that result in jun and fos protein induction in the skin in a manner that reduces or eliminates the expression of UVB-inducible MMPs.

Fig. 1 schematically represents the pathways of UVB-inducible MMP expression. As shown in Fig. 1, UVB exposure generates reactive oxygen intermediates (ROI) which stimulate AP-1 and NF- κ B activity, which in turn induces cytokines and growth factors. The interaction of those cytokines and factors with their receptors trigger the small GTP binding proteins RAC/CDC42 and RAS. Those proteins activate the three kinase cascades that are essential to production of the jun and fos proteins which make-up AP-1. AP-1 induces expression of certain MMPs. The agents that prevent photoaging can act on the MMPs, the transcription factors AP-1 and NF- κ B, and/or one or more of the molecules involved in the three kinase cascades shown in Fig. 1. Aspirin and E5510 (described by Fujimori, T., et al., *Jpn J Pharmacol* (1991) 55(1):81-91) inhibit NF- κ B activation. Farnesyl transferase inhibitors such as B-581 (described by Garcia A.M., et al., *J Biol Chem* (1993) 268(25):18415-18), BZA-5B (described by Dalton M.B. et al., *Cancer Res* (1995) 55(15):3295-3304), farnesyl acetate, and (α -hydroxyfarnesyl) phosphoric acid act on RAS and inhibit activation of the ERK cascade; whereas geranyl geranyltransferase inhibitors and lisofylline inhibit activation of the JNK cascade. Compounds such as SB202190 (described by Lee, J.C., et al., *Nature* (1994) 372:739-746) and PD98059 (described by Dudley, D.T., et al., *PNAS (USA)* (1995) 92:7686-7689) inhibit specific kinases in the cascades. Retinoids such as those disclosed in U.S. Pat. No. 4,877,805 and the dissociating retinoids that are specific for AP-1 antagonism such as those described by Fanjul, et al. (*Nature* (1994) 372:104-110), glucocorticoids, and Vitamin D3 target AP-1. Other retinoids, besides retinol, include natural and synthetic analogs of vitamin A (retinol), vitamin A aldehyde (retinal), vitamin A acid (retinoic acid, including all-trans and 13-cis retinoic acid), and other as described in EP 379367 A2. Finally, MMPs may be inhibited

by BB2284 (described by Gearing, A.J.H. et al., Nature (1994) 370:555-557), GI129471 (described by McGeehan G.M., et al., Nature (1994) 370:558-561), TIMPS, Galardin, Batimastat, and Marimastat, and hydroxamates, and other known inhibitors.

One or more of these MMP inhibitors are preferably administered topically to the skin that is to be exposed to sunlight. For such administration they will normally be formulated as creams, gels, ointments, sprays or lotions. Conventional pharmacologically and cosmetically acceptable vehicles may be used to formulate the inhibitor(s). Examples of such vehicles are described in U.S. Pat. No. 4,877,805 and EPA Pub. No. 0586106 A1. As indicated, one or more inhibitors may be present in a given formulation. For instance, a combination of inhibitors that act on two or more different molecules involved in effecting MMP degradation of the skin may be used. The formulations may also contain additives such as, emollients, skin permeation enhancers, pigments, and perfumes. In addition, the formulation may contain ingredients such as absorbent particles (e.g. polymer beads) that provide sustained release of the inhibitors to the skin. The weight concentration of inhibitor(s) in the formulation will usually be 0.01% to 10%, more usually 0.1% to 1%. Normally about 50 mg of formulation will be applied per cm² of skin.

The inhibitors are preferably applied to the undamaged skin prior to exposure to sunlight. The application regimen (i.e. daily, weekly, etc.) will primarily depend upon the longevity (e.g., metabolism, half-life in the skin) of the inhibitor(s) and the molecular targets of their action. It may also be effected by bathing, perspiration, and the extent of sunlight exposure. Usually they will be applied daily.

The invention is further illustrated by the following examples. These examples are not intended to limit the invention in any manner.

EXAMPLES

5 Determination of Molecular Basis of UVB-Induced Photoaging High UVB Dose Induction of MMPs

The time course of changes in MMP-1, MMP-3, MMP-9, and MMP-2 mRNA, protein, and enzymatic activity levels following UVB exposure were determined as follows.

10 Subjects were adult Caucasians (approximately equal numbers of males and females) with light to mild pigmentation. The UVB dose required to cause barely perceptible skin reddening (minimal erythema dose, or "MED") for each subject was determined 24 hours post
15 irradiation. One (1) MED for all subjects ranged from 30-50 mJ/cm². The subjects' buttocks were irradiated with 2 MED UVB with an Ultralite Panelite lamp containing four F36T12 ERE-VHO UVB tubes. Irradiation intensity was monitored with an IL443 Phototherapy Radiometer and a
20 SED240/UVB/W photodetector. UVB output, measured 48 cm from the source, was 0.5 mW/cm². For each subject skin was removed by keratome from four sites (one non-irradiated, three irradiated) at 8, 16, 24, 48 and 72 hours following irradiation. Tissue was snap frozen and total RNA
25 isolated and analyzed by Northern blot as described by Fisher, G.J. et al., J Invest Dermatol (1991) 96:699-707. Band intensities were quantified by PhosphorImager. Values for MMP transcripts were normalized to those for control gene 36B4. The results of these tests are shown
30 in Figs. 2a (MMP-1), 2b (MMP-3), 2c (MMP-9), and 2d (MMP--2). Results are means \pm SEM (n=6 for 8, 16, 48, and 72 hours and n=17 for no UVB control and 24 hours) and are presented as fold increase of normalized values relative to non-irradiated skin. The bands displayed in the
35 Figures are composites from several individuals.

As shown in Figs. 2a-d, induction of MMP-1, MMP-3, and MMP-9 mRNAs was maximal (6-60 fold) within 16 to 24 hours and returned to near baseline within 48 to 72 hours. MMP-2 mRNA was detectable, but only elevated 1.6-fold 24 hours post irradiation. Time courses for induction of MMP-1 and MMP-9 proteins and activities by 2 MED UVB paralleled those observed for their mRNAs. Neither MMP-2 protein nor activity was induced.

Northern analysis of UVB-treated skin with a MMP-3 (stromelysin I)-specific probe yielded results identical to those obtained with a full-length MMP-3 probe (Fig. 2b), while hybridization with a MMP-10 (stromelysin II)-specific probe yielded no signal. This indicates that among the stromelysins, UVB induces predominantly stromelysin I.

Low Dose UVB Induction of MMPs

Subjects were exposed to UVB doses ranging from 0.01 to 2 MED as described above. Full thickness skin samples (6mm cylinders) were obtained 24 hours after irradiation from treated and untreated sites. The samples were homogenized in 20mM Tris HCl (pH 7.6), 5mM CaCl₂, and centrifuged at 3000xg for 10 minutes. Supernatants were used to measure MMP-1 and MMP-9 proteins by Western blot (100 µg/lane), using chemiluminescence detection and activity by hydrolysis of ³H fibrillar collagen (100 µg/assay) according to Hu, C.L. et al., Anal Biochem (1978) 88:638-643 and gelatin zymography (20 µg/assay) according to Hibbs, M.S. et al., J Biol Chem (1985) 260:2493-2500, respectively. The MMP-2 and MMP-9 antibodies used are described by Werb, Z. et al., J Cell Biol (1989) 109:877-889 and Murphy, G. et al., Biochem J (1989) 258:463-472, respectively. The results of these tests are shown in Figures 3a and 3b.

In Fig. 3a, MMP-2 protein values are shown by the open bars whereas MMP-2 activity values are shown by the

cross-hatched bars. The Fig. 3a inset shows representative Western blots from two subjects. The larger 54 KDa band is intact MMP-2 and the smaller 45 KDa band is the proteolytically processed activated form of MMP-2.

In Fig. 3b MMP-9 protein values are shown by the open bars where MMP-9 activity values are shown by the cross-hatched bars. The Fig. 3b inset shows a representative Western blot (left panel) and a representative zymogram (right panel). Multiple bands on the zymogram are proteolytically processed active forms of MMP-9.

Band intensities were quantified by laser densitometry. Results are given as means \pm SEM of $n=10$.

As shown in Figs. 3a and 3b, induction of MMP-2 and MMP-9 proteins and activities was dose dependent, and for both MMPs changes in protein and activity mirrored each other. MMP-2 was induced by all doses of UVB tested, while MMP-9 was induced by doses ≥ 0.1 MED. Induction was maximal at one (1) MED and approximately half maximal at 0.1 MED. 0.1 MED UVB is equivalent to two to three minutes solar irradiation on a summer day, which causes no perceptible skin reddening.

Low Dose UVB Induction of AP-1 and NF- κ B

Subjects were irradiated and tissue samples taken as described above. Nuclear extracts were prepared from the samples as described by Fisher, G.J. et al., J Biol Chem (1994) 269:20629-20635. Biopsies (approx. 200 mg wet weight) containing $\sim 10^8$ cells yielded 500 μ g nuclear extract protein, on average. Electrophoretic mobility shift assays (8 μ g nuclear extract protein) were performed using 32 P-labeled DNA probes containing AP-1 and NF- κ B consensus and mutated DNA-binding sequences as described by Fisher, G.J. et al., *supra*. Antibodies for supershifts were obtained from Santa Cruz Biotechnology. Jun and fos antibodies had broad reactivity to all jun and fos family

members, respectively. NF- B antibody was specific for p65/Rel A. The results of these assays are shown in Figs. 4a, 4b, 4c and 4d (NS designates non-specific examples). The insets for these Figs. show representative AP-1 and NF- B retarded complexes. +Compet designates addition of 100-fold excess unlabelled probe; Mut designates mutated ^{32}P probe.

Fig. 4a depicts AP-1 and NF- B binding in non-irradiated and irradiated (four hours after 2 MED UVB) skin. As shown in Fig. 4a binding of both transcription factors to their DNA response elements was specific as demonstrated by loss of retarded complexes with mutated labeled probes. Antibody supershifts demonstrated that the specific AP-1 and NF- B retarded complexes observed with extract from UVB-irradiated skin contained jun and fos proteins, and Rel A protein, respectively.

Figs. 4b and 4c show the time courses of induction of AP-1 and NF- B DNA binding, respectively, by 2 MED UVB. The results reported are means \pm SEM, n=9. As shown, induction of both factors occurred within 15 minutes.

Fig. 4d shows the dose dependence of induction of AP-1 (represented by open bars) and NF- B (represented by cross-hatched bars). DNA binding was measured 30 minutes after irradiation. As shown half maximal induction of both factors occurred at approximately 0.1 MED and maximal induction occurred at one (1) MED. The UVB dose dependencies for induction of these factors closely matched those reported above for induction of MMP-2 and MMP-9, consistent with the participation of these transcription factors in the UVB-induced increases in these two MMPS.

Inhibition of UVB Induction of AP-1, MMP-2 and MMP-9

0.1% all-trans retinoic acid (t-RA) and its vehicle (70% ethanol and 30% propylene glycol) or 0.05% of the glucocorticoid (GC) clobetasol propionate and its vehicle

(2% propylene glycol plus 2% sorbitan sesquioleate in white petrolatum) were applied (300 mg formulation/6 cm² skin) to subjects for 48 hours as described by Fisher, G.J. et al., J Invest Dermatol (1991) 96:699-707. Treated skin sites were then irradiated with 2 MED UVB. Skin was obtained as described above 30 minutes after exposure to AP-1 measurements or 24 hours after exposure for MMP measurements. AP-1 measurements and MMP-1 and MMP-9 measurements were made as described above. To determine whether t-RA altered UVB-induced skin reddening, subjects were treated with 0.1% t-RA and its vehicle for 24 hours. Treated areas were irradiated with 10-80 mJ/cm² UVB and skin reddening determined 24 hours after by a Minolta chromameter. The results of these tests are shown in Figs. 5a, 5b, 5c, 5d and 5e.

Figs. 5 a reports the AP-1 measurements. As shown pretreatment of skin with t-RA reduced UVB-induced AP-1 DNA binding by approximately 70%.

Figs. 5b and 5c report the MMP-1 and MMP-9 measurements. As shown, t-RA pretreatment reduced UVB-induced MMP-1 and MMP-9 mRNAs, proteins and activities 50%-80%.

Fig. 5d reports tests on the effect of t-RA pretreatment on skin reddening. As shown, although t-RA absorption overlaps the UVB range (t-RA max = 351 nm), t-RA did not reduce UVB-induced skin reddening. This indicates that the observed reductions in AP-1 and MMP induction were specific rather than due to absorption of UVB by t-RA.

Fig. 5e reports the effects of pretreatment of the skin with GC. As shown GC pretreatment reduced MMP-1 and MMP-9 activities to extents similar to those observed from t-RA pretreatments.

The publications referred to in the above specification are hereby expressly incorporated by reference.

We claim:

1. A method for inhibiting photoaging of unphotodamaged skin of a human due to exposure of the skin of the human to ultraviolet B irradiation (UVB), comprising: providing at least one inhibitor of (a) the activity of UVB irradiation inducible MMPs in the skin, (b) one or both of the transcription factors AP-1 and NF- B and (c) at least one of the GTP binding proteins or kinases involved in the activation and/or production of jun or fos proteins that comprise AP-1; and topically administering said inhibitor to the skin prior to such exposure in an amount sufficient to inhibit the production or activity of UVB-inducible MMPs, one or both of AP-1 and NF- B, or least one of the GTP binding proteins or kinases involved in the activation and/or production of jun or fos proteins.

2. The method of claim 1 wherein said method inhibits photoaging induced by exposure to a dose of UVB below the minimum dose required to cause reddening of said skin.

3. The method of claim 2 wherein said UVB dose is above about 5 mJ/cm².

4. The method of claim 1 wherein the inhibitor inhibits the activity of at least one of AP-1 and NF- B.

5. The method of claim 1 wherein the inhibitor inhibits the activity of UVB-inducible MMPs.

6. The method of claim 1 wherein the inhibitor inhibits a GTP binding protein or kinase essential to the production of jun or fos proteins.

7. The method of claim 4 wherein the inhibitor inhibits AP-1 and is a retinoid, a glucocorticoid, or Vitamin D3.

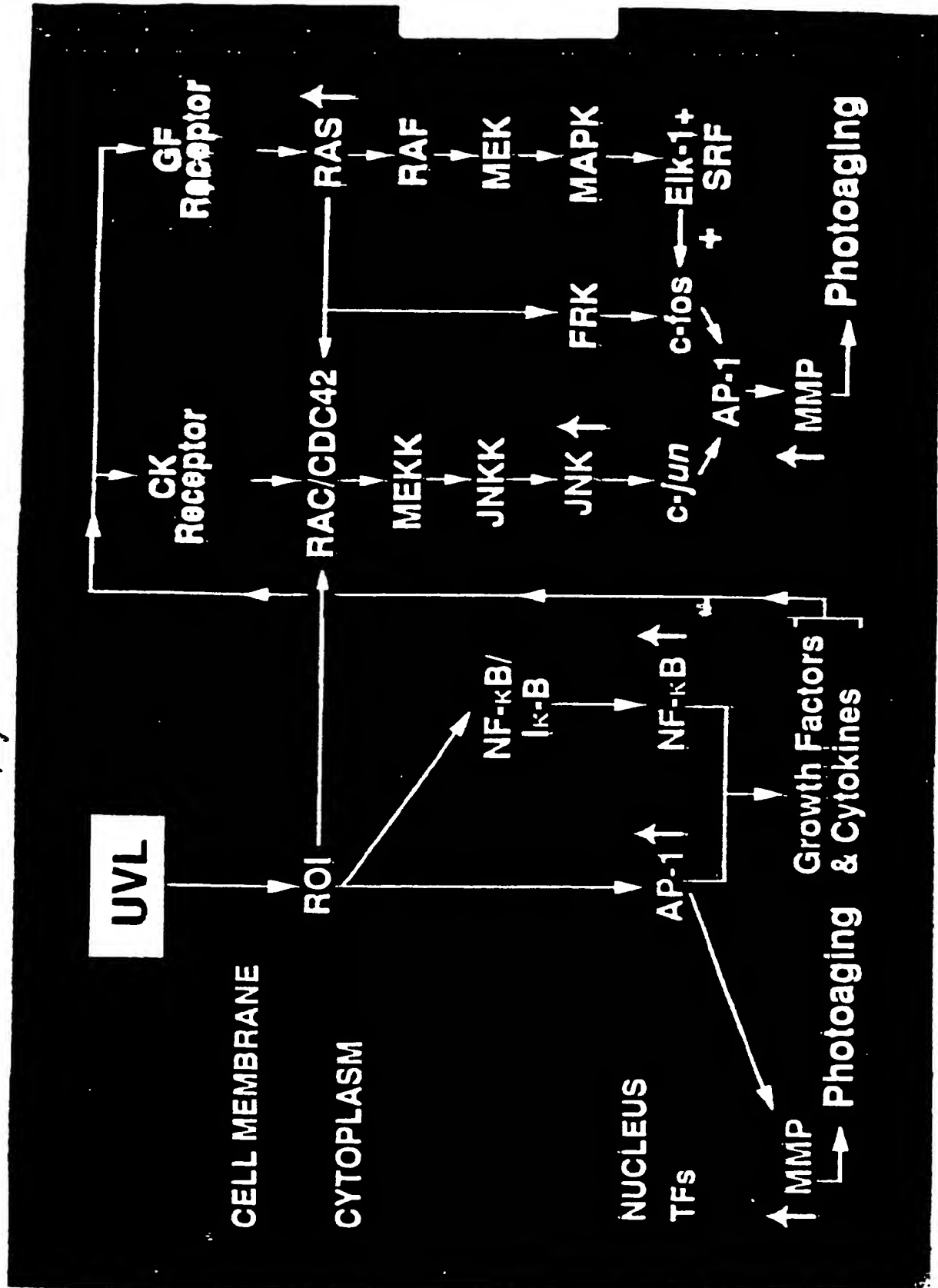
5 8. The method of claim 4 wherein the inhibitor inhibits NF- B and is a glucocorticoid, aspirin or E5510.

9. The method of claim 5 wherein the inhibitor is a TIMP, Galardin, Batimastat, Marimastat, or a hydroxamate.

10 10. The method of claim 6 wherein the inhibitor is a farnesyl transferase inhibitor, a geranyl geranyltransferase inhibitor, SB202190, or PD98059.

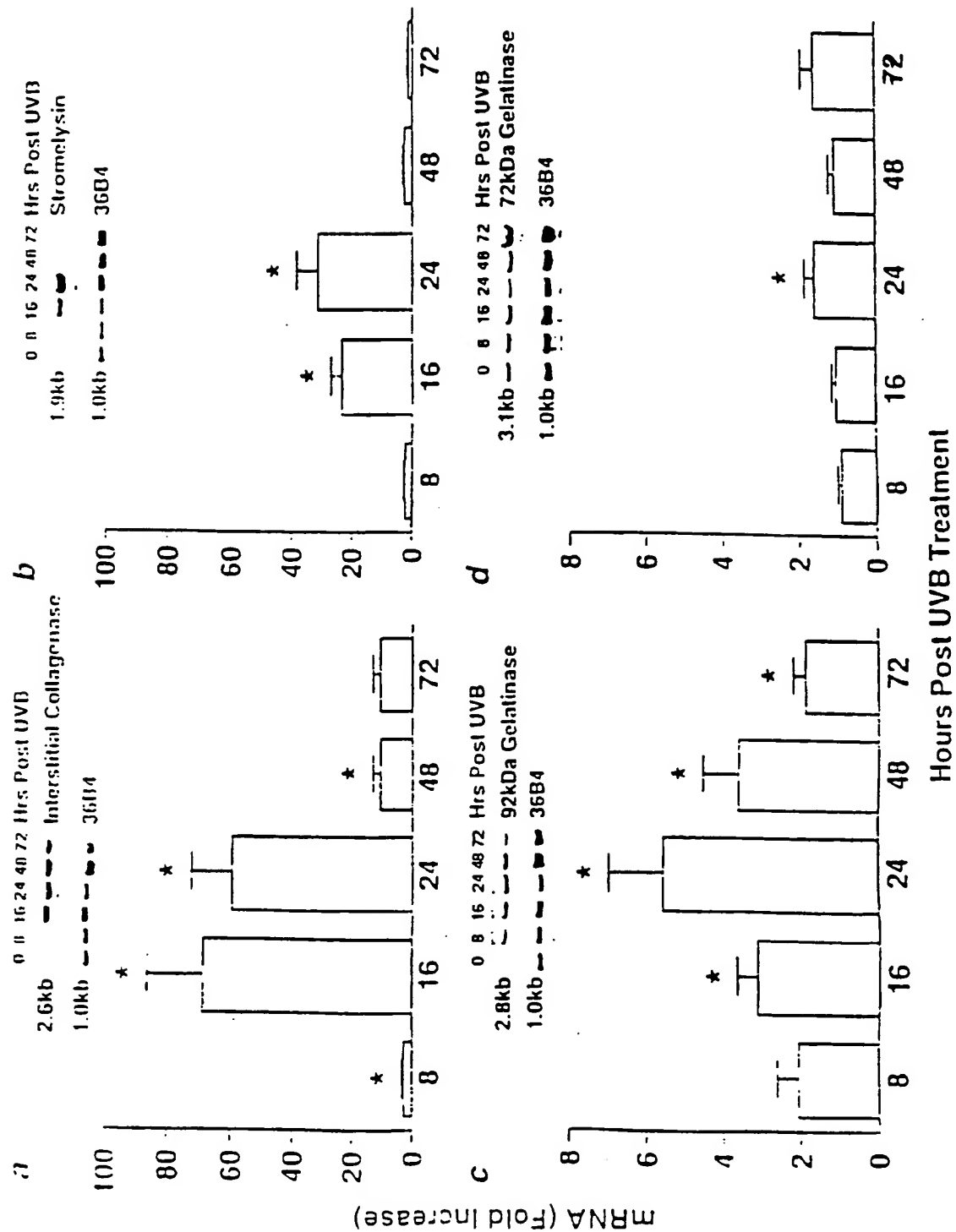
11. The use of an inhibitor of an ultraviolet B irradiation inducible matrix metalloproteinase in the manufacture of a medicament for preventing photoaging of undamaged human skin.

Figure 1



2 / 8

Figure 2



3 / 8

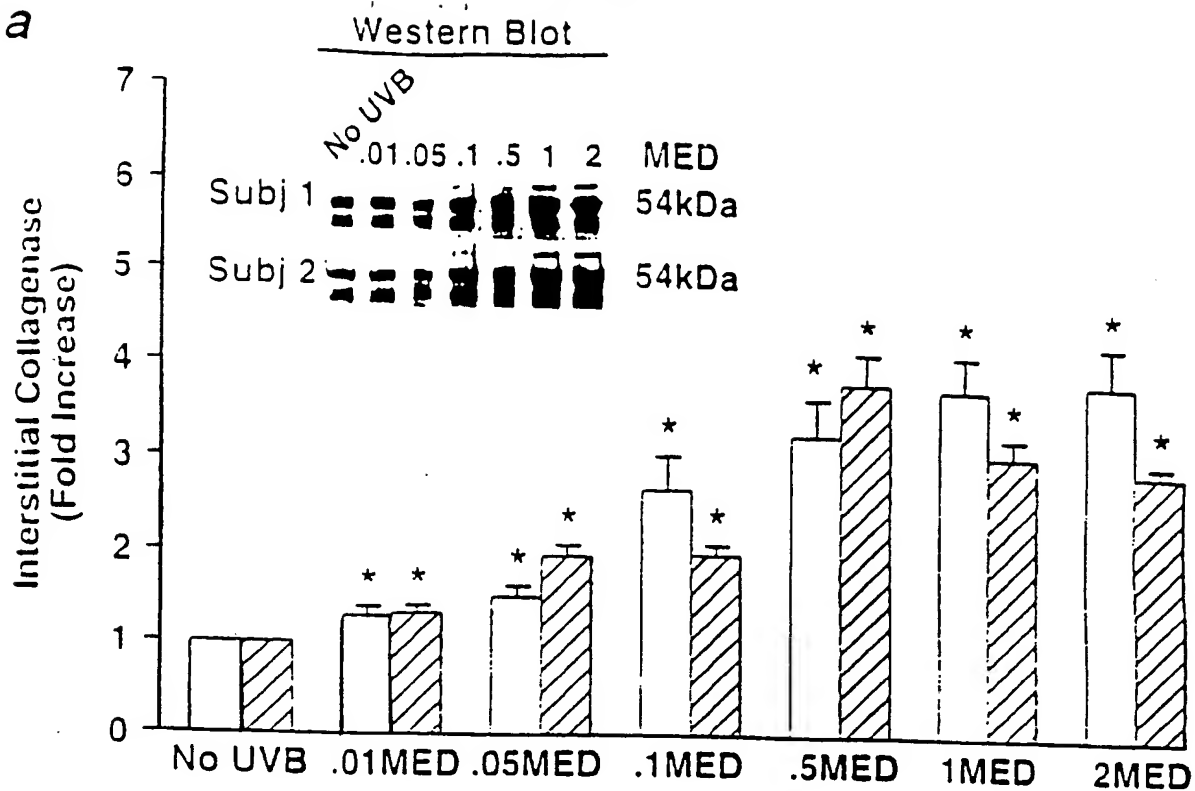
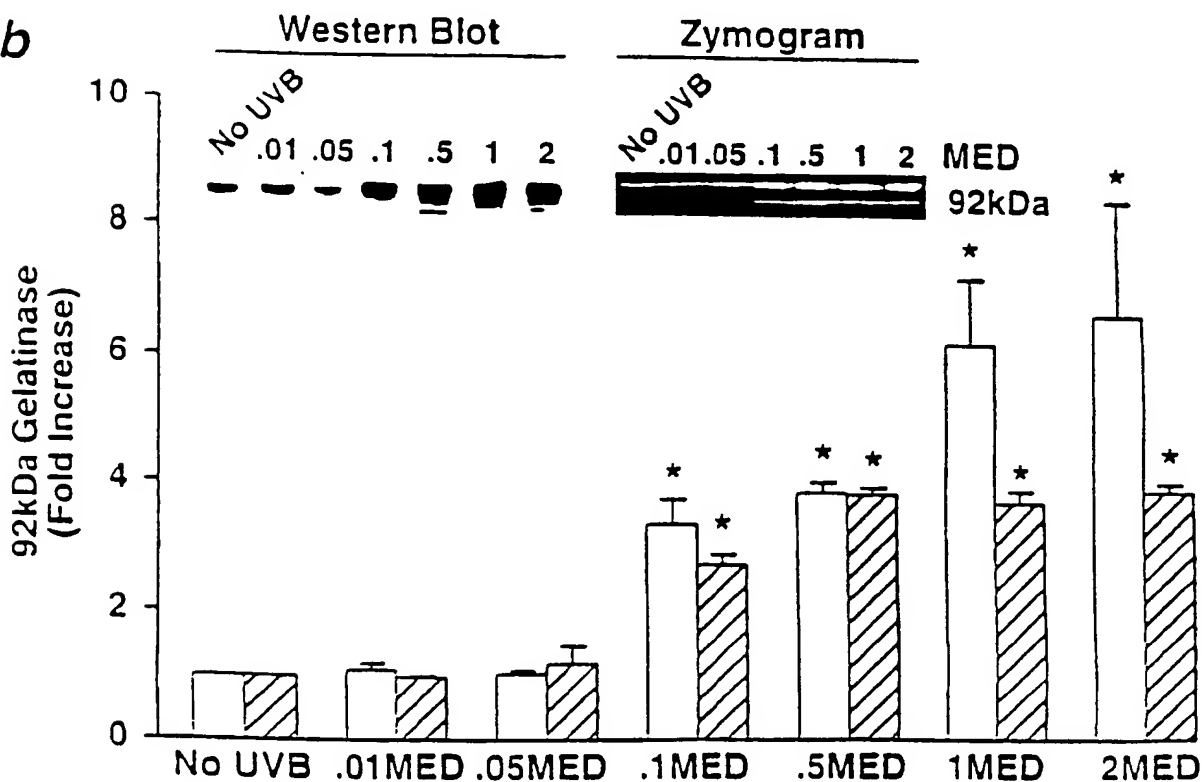
a**b**

Figure 4

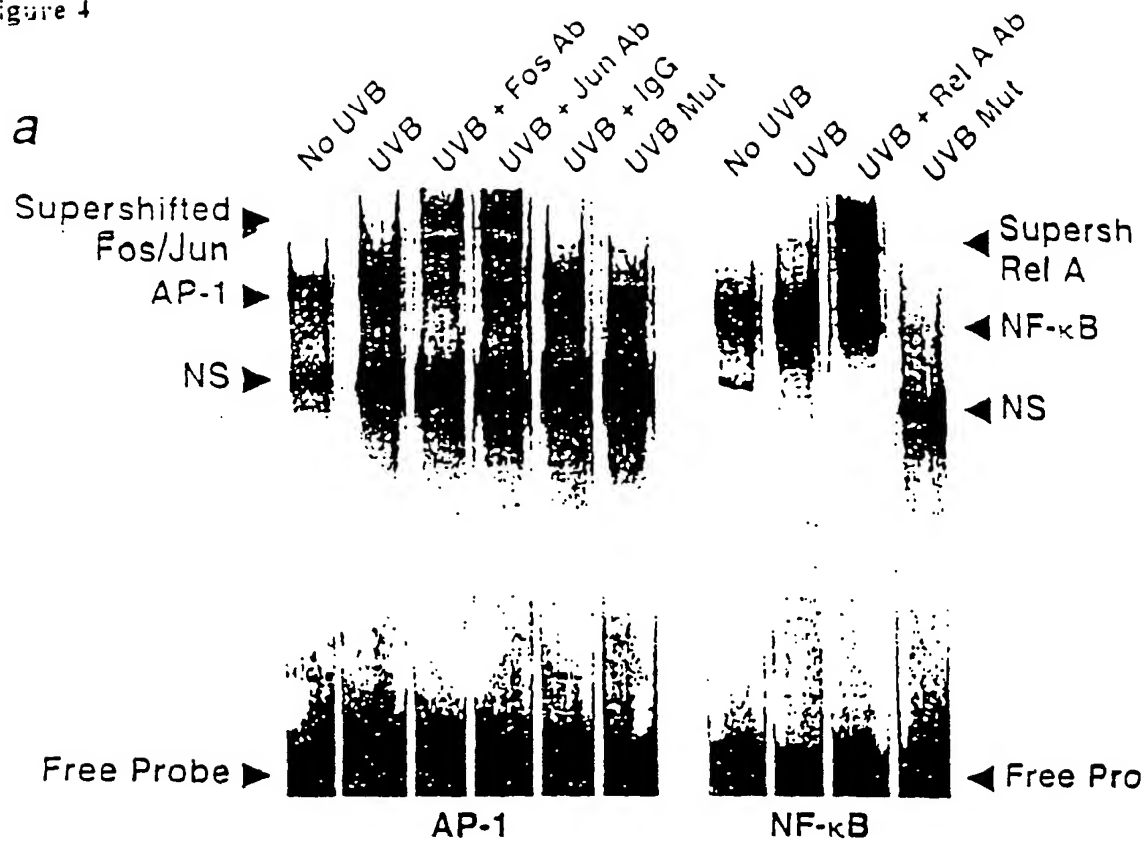
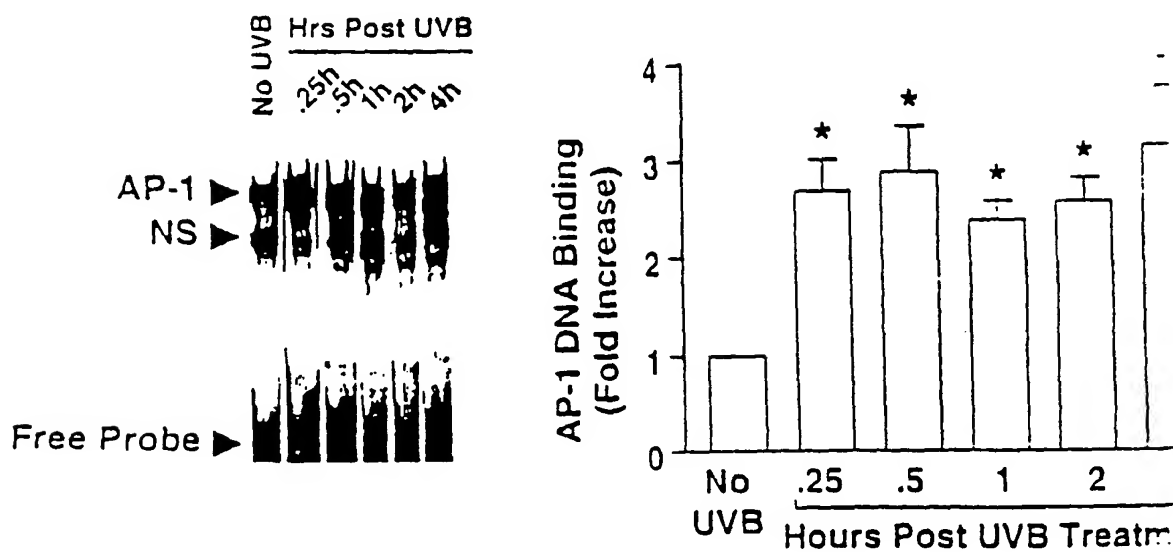
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Figure 4

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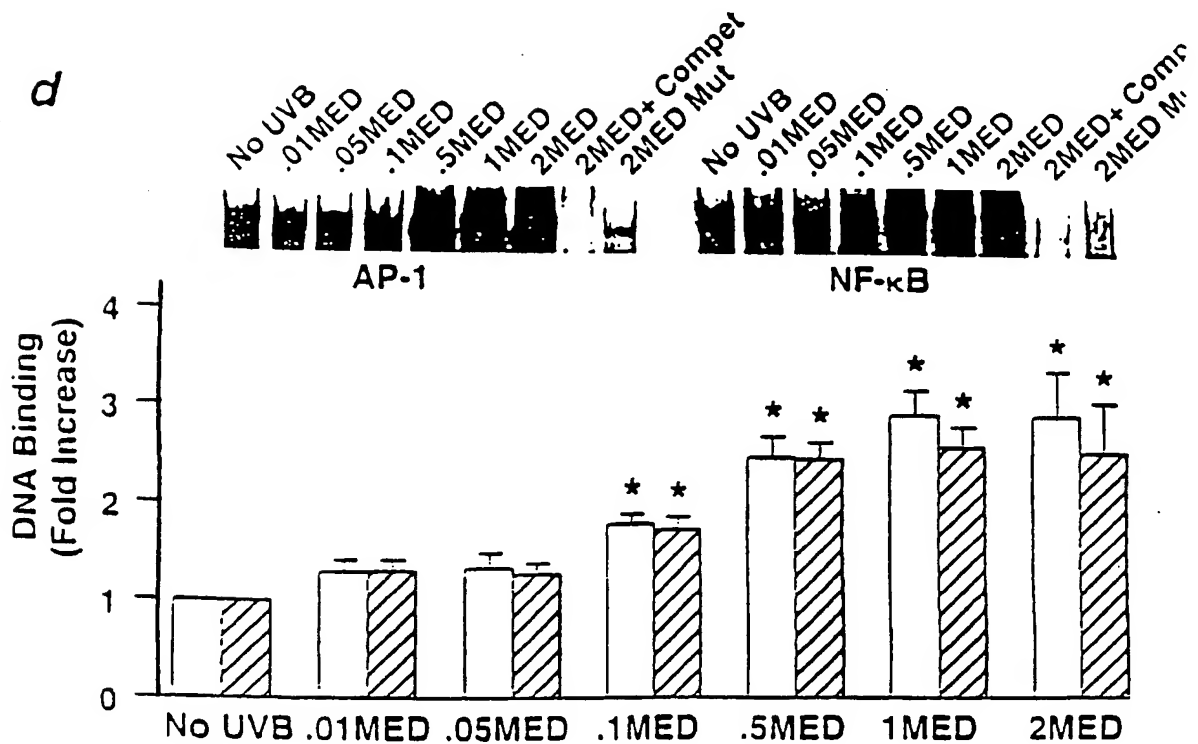
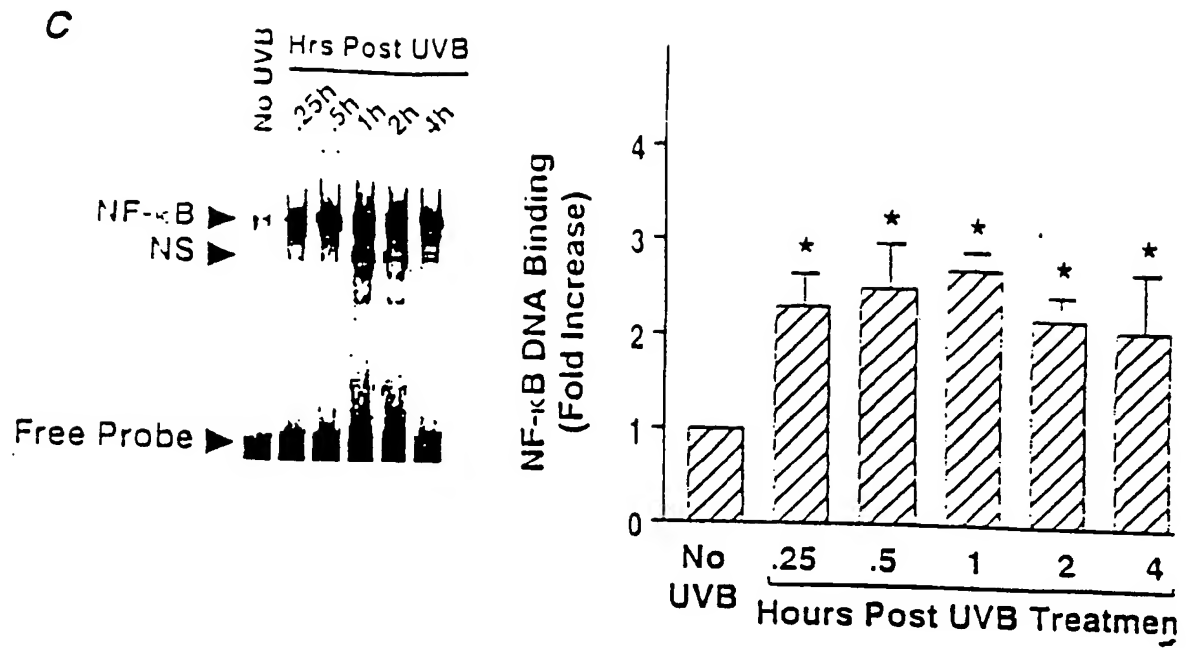
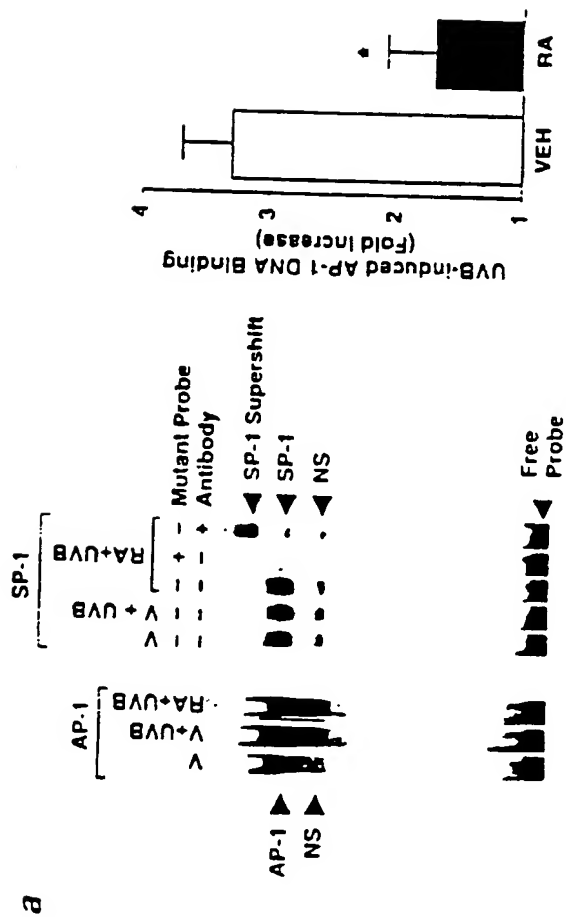
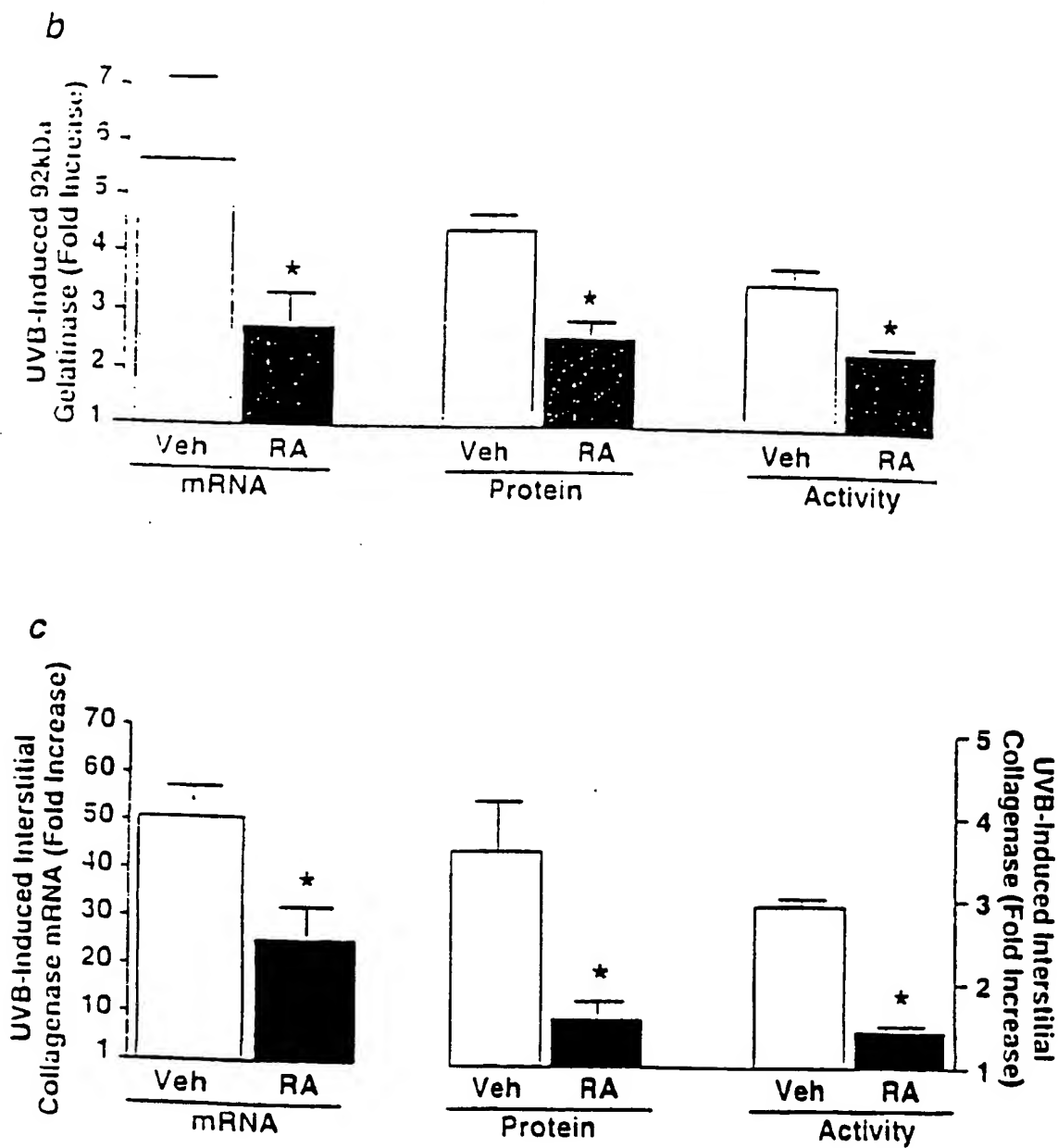


Figure 5



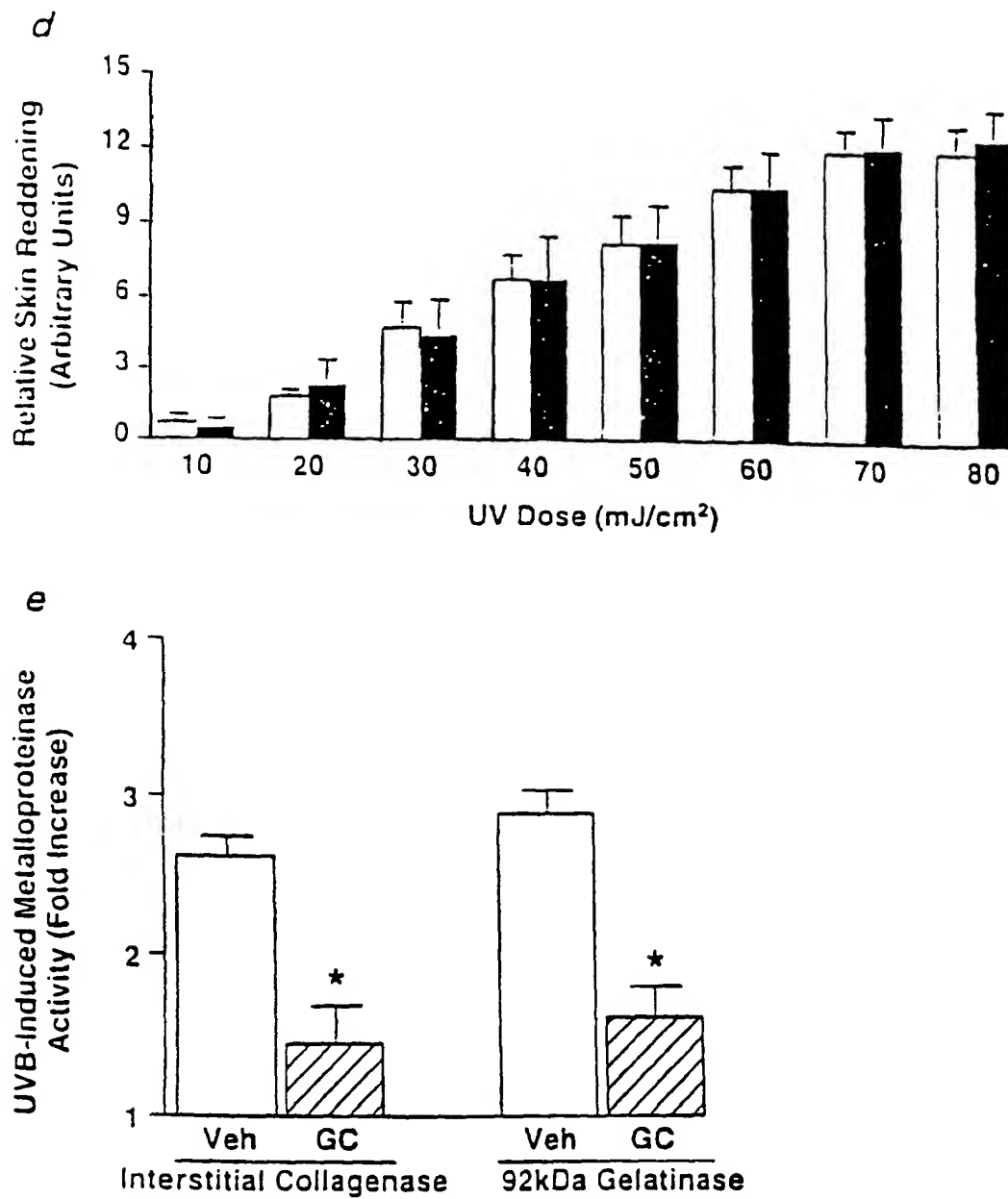
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Figure 5



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Figure 5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/00791

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 7/40, 7/42, 31/59

US CL : 424/59, 60, 167

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/59, 60, 167

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONEElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,877,805 A (KLIGMAN) 31 October 1989, see entire document.	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

26 MARCH 1997

Date of mailing of the international search report

07 APR 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

SHELLEY A. DODSON

Telephone No. (703) 308-1235

